

Figure S1. Maximum parsimony phylogenetic tree of *kitl* ORF sequences. Mouse *kitl* was used as an outgroup. Bootstrap support values from 100 replicates are shown. Accession numbers of sequences: guppy *kitla*, KC143125; mouse *kitl*, NM_013598; zebrafish *kitla*, AY929068; zebrafish *kitlb*, AY929069.

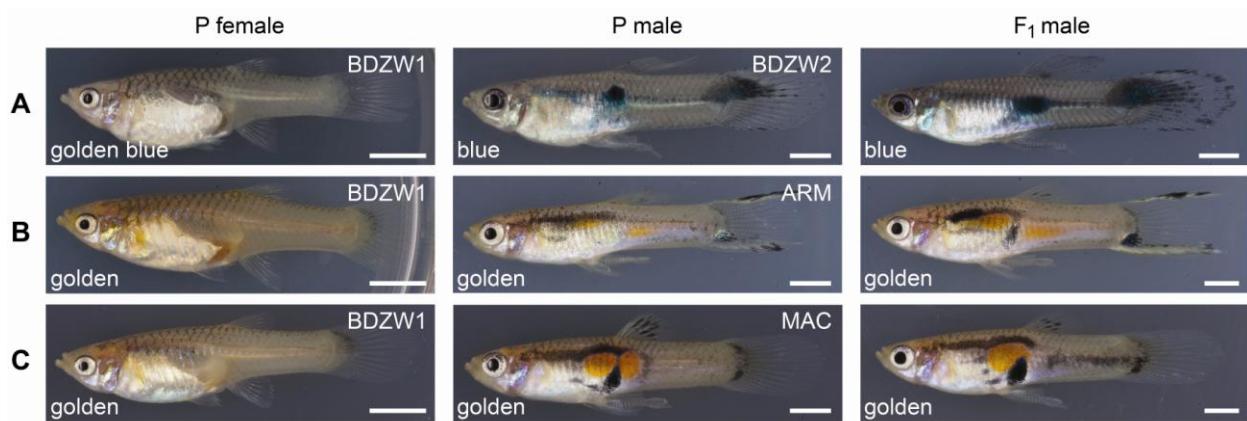


Figure S2. Complementation analyses.

(A) Non-complementation of the blue phenotype. All F₁ from a cross between a golden blue BDZW1 female and a blue BDZW2 male were blue. (B,C) Non-complementation of the golden phenotype. All F₁ from crosses of golden BDZW1 females to golden ARM and MAC males were golden. Parents (P) and representative F₁ male are shown for each cross. Scale bars: males 2 mm; females 5 mm.

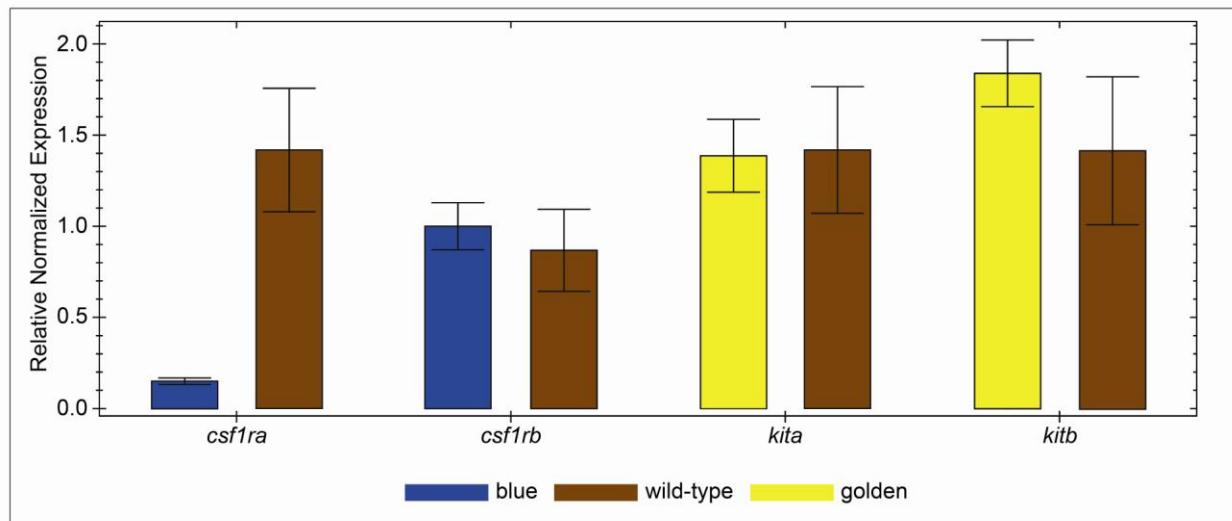


Figure S3. Expression levels of *csf1ra*, *csf1rb*, *kita*, and *kitb* in adult female skin.

Normalized expressions ($\Delta\Delta C_q$) of the genes in wild-type, golden, and blue female skin are shown. Expression levels were determined by real-time quantitative PCR using three biological replicates (one replicate refers to skin of one female) with three technical repetitions each. Expression was normalized to *gapdh* expression. Primer efficiencies were: *gapdh*, 85.3%; *csf1ra*, 96.4%; *csf1rb*, 97.6%; *kita*, 98.2%; *kitb*, 95%.

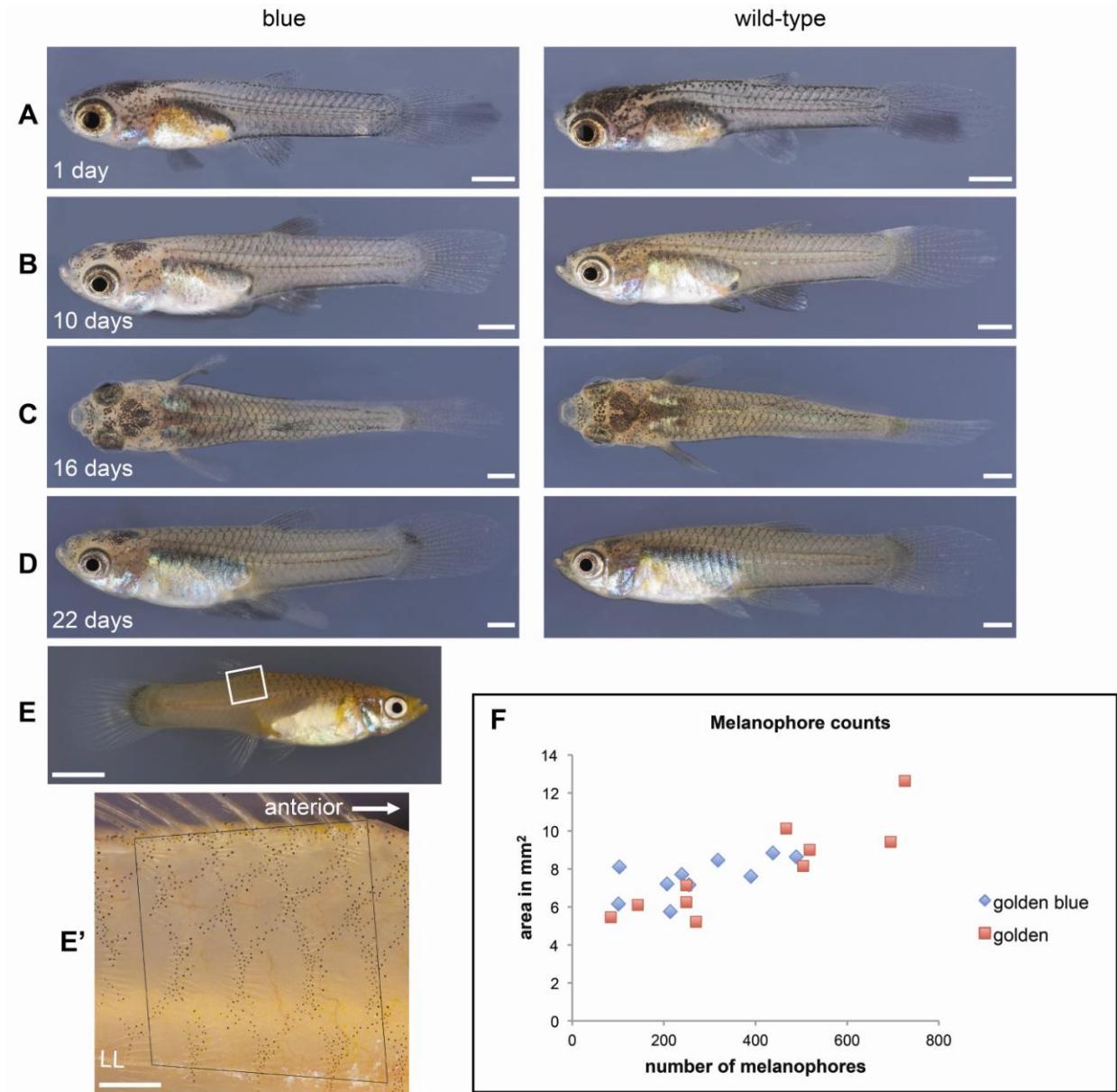


Figure S4. Melanophore pattern development in wild-type and *blue* mutant fish.

(A-D) Photos of the same males over a three-week time course (days are after birth). The blue fish has a BDZW2, the wild-type fish a BDZW1 background. The amount of melanophores of *blue* mutant and wild-type newborns was not compared, as variation in melanophore number is high between guppy strains (unpublished observation). (E') Detail of area boxed in (E); black rectangle indicates area below dorsal fin in which melanophores were counted. (F) Melanophore counts in 10 *golden* and 10 *golden blue* mutant females. The number of melanophores is strongly correlated with the size of the area. All fish shared the same grandparents and were approximately one year old. LL: Lateral line. Scale bars: (A-D) 1 mm; (E) 5 mm; (E') 1 mm.

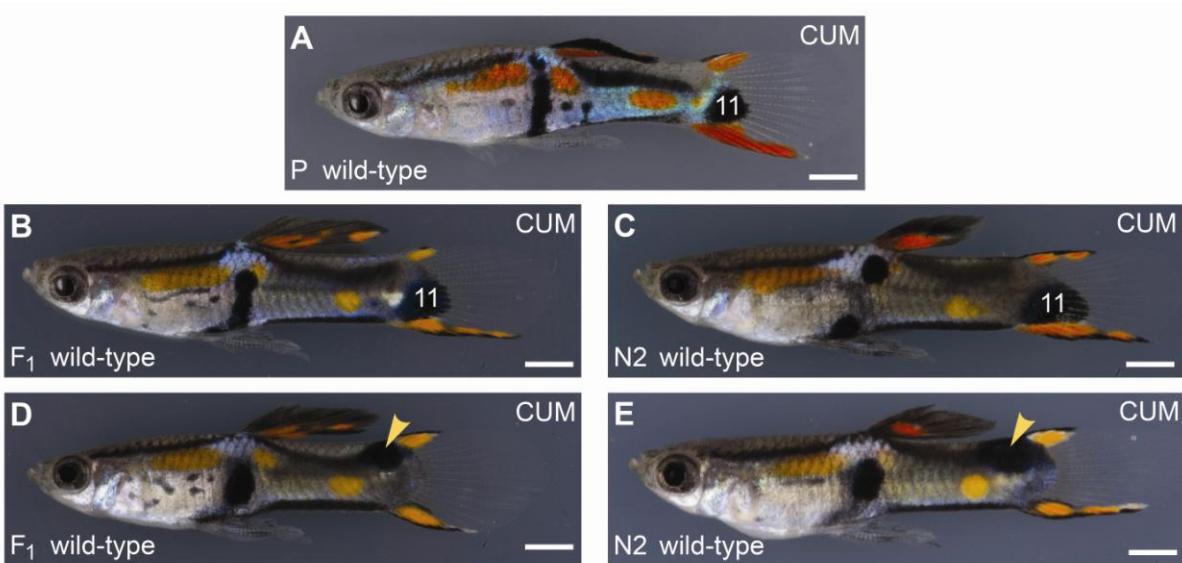


Figure S5. Inheritance of the ventral black spot on the tail fin of the Cumaná strain. (A) Grandfather, (B) F₁, and (C) N2 males with a ventral black spot on the tail fin (11). (D) F₁, and (E) N2 males with a dorsal black spot on the caudal peduncle (yellow arrowheads). The black spot on the tail fin was present in 33 of 55 F₁ males; all other males had a black spot on the caudal peduncle. This suggests that cofactors exist that modulate this trait, which might be derived from autosomes or the X chromosome of the BDZW1 strain in this case. All N2 males had the spot at the same position as their father. (C) and (E) are the sons of (B) and (D), respectively. P, grandfather. Scale bars: 2 mm.

File S1

Photos tracking the development of the melanophore pattern after birth.

Image names include image number and days after birth at which the image was taken. Additional pictures with higher magnification of some of the fish are available upon request. Note that the gestation time in the guppy varies; although all fish were imaged at identical time points after birth, they might not be in the exact same developmental stage. We did not take any photos from the dorsal aspect during the first week after birth, as most fish did not survive this at this age. Dataset was deposited at: ftp://ftp.tuebingen.mpg.de/ebio/csf1ra_kita_mutants/

File S2

Photos of males taken from backcrosses to investigate the influence of the golden and blue mutations on the male-specific ornaments.

Cross20_BDZW1_CUM: cross between a golden blue BDZW1 female and a wild-type Cumaná male. Seven F1 males were backcrossed to golden blue BDZW1 females (BC1 to 7 pairs) to produce a N2 generation. The color pattern of one of the golden males (cross20_F1fBC6_whitem_0488) resembled the one of the BDZW1 strain rather than the one of the Cumaná strain, which might be the outcome of a rare recombination event. Cross21_BDZW1_GU: cross between a golden blue BDZW1 female and a wild-type Guanapo male. Four F1 males were backcrossed to golden blue BDZW1 females (BC1 to 4 pairs) to produce a N2 generation. Cross23_BDZW1_QU: cross between a golden blue BDZW1 female and a wild-type Quare6 male. Four F1 males were backcrossed to golden blue BDZW1 females (BC1 to 4 pairs) to produce a N2 generation. Cross24_BDZW1_QUII: cross between a golden blue BDZW1 female and a wild-type Quare6 family II 215-3 male. Four F1 males were backcrossed to golden blue BDZW1 females (BC1 to 4 pairs) to produce a N2 generation. Image names include cross number, generation, phenotype, sex, and identification number. P, grandparents; BCs, backcrosses; m, male; f, female; F1fBC1, F1 offspring (here called N2 generation) from BC1 pair; wtm, wild-type male; whitem, golden male; bluem, blue male; ghostm, golden blue male. Dataset was deposited at: ftp://ftp.tuebingen.mpg.de/ebio/csf1ra_kita_mutants/

Table S1 Methods used for full-ORF amplification of pattern formation candidate genes

Gene	Origin of partial sequences	Primers for RACE PCR	Primers used for full-ORF amplification by PCR	Primers used for internal sequencing of full-ORF clones
	Consensus primers:			
		3'-RACE PCR primers (forward):		
<i>Kitla</i>	Forward: 5'-AAGTT GGATACGCGTCTG TGTCC-3'	5'-CTCATCAGCTCGTTGCCAA GTC-3' (reaction 1)	5' UTR forward: 5'-ATTGGATAT GTGCACACAGGATGATGAC-3'	-
	Reverse: 5'-CTTCC ACACMAGCAGGA AWA- 3'	5'-CACTGCTGGCCCTAACATTCTG-3' (reaction 2)	3' UTR reverse: 5'-GTTTGCTTT TCACCTTTGAACACCAACAG-3'	
	We amplified a short piece of the guppy ortholog of <i>kitla</i> by using consensus primers that were chosen based on an alignment of <i>kitla</i> of other teleost species ^a . We then designed guppy-specific <i>kitla</i> primers for RACE PCR; however, the 5'-RACE PCR failed. To obtain a part of the guppy <i>kitla</i> 5' UTR, we ordered forward consensus primers in the 5' UTR of <i>kitla</i> based on an alignment of <i>kitla</i> sequences of other teleost species ^b . 5'-ATTGGATATGTGCACACAGGATGATGAC-3' was used successfully in combination with guppy-specific <i>kitla</i> reverse primers. Subsequently, we used this primer in combination with a reverse primer in the 3' UTR for full-ORF amplification.			
<i>Kita</i>	<i>Kita</i> marker sequence (Tripathi et al., 2008)	3'-RACE PCR primers (forward): 5'-CAGAGCTGAAGGTCTCAGTT ACTTGG-3' (reaction 1) 5'-TCAGCACCTGTGAGCTAAAGG AGAAC-3' (reaction 2)	5' UTR forward: 5'-AGAGCT ACACCAGCTTGACCAC-3' 3' UTR reverse: 5'-CGTAAC ATCACAGGCACTTAGAGG-3'	5'-GACAGTAAAGAAAGTCC CTTTAGGTCC-3' (forward) 5'-GGAACCTCCACTGGTGAT TGTAG-3' (reverse) 5'-GACAAGTCGTCTCATCG AACATCTC- 3' (reverse)
	We used a partial <i>kita</i> sequence to design primers for RACE PCR. The 5'-RACE PCR failed; to obtain a part of the guppy <i>kita</i> 5' UTR, we ordered forward consensus primers in the 5' UTR of <i>kita</i> based on an alignment of <i>kita</i> sequences of other teleost species ^c . 5'-CTSYAGGAC AACAGCATGYTTG-3' was used successfully in combination with guppy-specific <i>kita</i> reverse primers. Subsequently, we designed primers in the UTRs for full-ORF amplification.			
<i>Csf1ra</i>	Transcriptome database	5'-RACE PCR primers (reverse): 5'-CTATCCGTACATATTCTCATCTG TCTCC-3' (reaction 1) 5'-CAGTGTAGAAGCACTTGTAC GTTCC-3' (reaction 2)	5' UTR forward: 5'-CCTCCTCA AGCTGAAGACATC-3' 3' UTR reverse: 5'-ACCGACTCA GCCTTGAAC TG-3'	5'-GATCTGAGGTGTGAAGG CAAC -3' (forward) 5'-CCTTCACAACATAGTTGG AGTCATTC- 3' (reverse) 5'-GTCTAGACATCCAGAGCA TCCTGAC- 3' (forward)

We detected an incomplete *csf1ra* transcript in our guppy transcriptome database (SHARMA, E., A. KÜNSTNER, B. A. FRASER, M. HOFFMANN, V. A. KOTTLER, G. ZIPPRICH, D. WEIGEL, and C. DREYER, unpublished data) and carried out a nested 5'-RACE PCR to obtain the missing 5' end of the wild-type *csf1ra* transcript. Based on the full-length cDNA sequence of *csf1ra*, we designed primers in the UTRs for full-ORF amplification.

Rapid amplification of cDNA ends (RACE)

First-strand cDNA for 5'/3'-RACE PCR was prepared with BD SMART PCR cDNA Synthesis Kit (Clontech) according to the manufacturer's instructions. The RACE PCR product of reaction 1 was diluted 50-fold to serve as template in reaction 2 (nested RACE PCR). Reverse (5'-RACE PCR) and forward (3'-RACE PCR) gene-specific primers were combined with the forward (5'-RACE PCR) and reverse (3'-RACE PCR) primer of the BD SMART PCR cDNA Synthesis Kit, respectively. Both PCR reactions were carried out with Advantage 2 Polymerase Mix (Clontech) as described before.

Accession numbers

^aGenBank EU218897 (stickleback, *Gasterosteus aculeatus*); Ensembl ultracontig72 (medaka, *Oryzias latipes*)

^bGenBank EU218897 (stickleback), AB285216 (fugu, *Takifugu rubripes*), AY929068 (zebrafish, *Danio rerio*), FJ907418 (goldfish, *Carassius auratus*); Ensembl GSTENT10006132001 (tetraodon, *Tetraodon nigroviridis*)

^cEnsembl MEDAKA1:4:1429800:1459632:1 (medaka), FUGU4:scaffold_13:1529100:1545623:1 (fugu)

Literature cited

TRIPATHI, N., M. HOFFMANN and C. DREYER, 2008 Natural variation of male ornamental traits of the guppy, *Poecilia reticulata*. Zebrafish 5: 265-278.

Table S2 List of primers used for real-time quantitative PCRs

Gene	Primers	Efficiency
<i>Gapdh</i>	Forward: 5'-ACATCAAGAAGGTTGTAAAGCTG-3' Reverse: 5'-ATCAAAGATGGAGGAGTGAGAAC-3'	85.3%
<i>Csf1ra</i>	Forward: 5'-AACTGGAGGAGGAGCAGGTAATC-3' Reverse: 5'-GTGACACTTAGGCTTGTACAG-3'	96.4%
<i>Csf1rb</i>	Forward: 5'-TTGACGAGTGACATGTTGCCTC-3' Reverse: 5'-ATCATCATCCTCTTCTGCTCTG-3'	97.6%
<i>Kita</i>	Forward: 5'-AAGAGAGCTGCAGATGGTGAC-3' Reverse: 5'-CGTCAGCTTCGAGGTACAGG-3'	98.2%
<i>Kitb</i>	Forward: 5'-ACCAAGAACGTGTACCTGACTC-3' Reverse: 5'-CTACACACAGAGCTAACCCCTCC-3'	95.0%

Table S3 List of primers used for genomic PCRs

Gene	Primers	Purpose	Remarks
<i>Kita</i>	Forward exon 6: 5'-TGTCTCTGAACG TTAGCATGGAG-3' Reverse in 36 bp 'exon' of insert: 5'-AAGCTTGTCAGGAATCTGGTATGG-3'	Amplification of <i>kita</i> ^{insert} (quick PCR test)	
<i>Kita</i>	Forward exon 6: 5'-TGTCTCTGAACGTTAGCATGGAG-3' Reverse exon 7: 5'-ACACGGAGAACATAGTTGGAGTCATT-3'	Amplification of <i>kita</i> ^{wt} (quick PCR test)	Elongation time of 2 min not sufficient to amplify <i>kita</i> ^{insert}
<i>Csf1ra</i>	Forward exon 16: 5'-ACATTGACGACCTGCTGAGATT-3' Reverse exon 17: 5'-CCTTCACAACATAGTTGGAGTCATT-3'	Amplification of <i>csf1ra</i> ^{wt} and <i>csf1ra</i> ^{indel}	

Table S4 Sequences and description of six *kita* splice variants found in *golden* mutants

Sequences of variants (V) ^a	Description	Predicted protein
>V1 gatgtggagttacaatgcgttagccggaaacgacaaaggaaacagtacgtatgtccgttggtggacgtt tatgaacggggtttatcagctcagcagaatccacaacagaaccctcacgtccgtcgccgacagttgt ctctgaacgttagcatggaggcgatccaaagccgcgcgcctctggacttgtctgttactccaataat ctcacataccataccagattccgtacaagcttcatggcgagaaccctcagaacacaccaggcaccacgtcat caccacgcacaggcaggactacatcagcggacttgtggacttgtggcgctaaacgcacagaagggt gcatttataccctcaagccctcaacggcgacgcgcggtaaaggcagaacttccgtttttatcgtaaag cctgcgtatagatcatggggcccactgtggatggacagggtgcactgtgtggctgaaggctaccctcccc agataaaatgttactactgcgagaagcatgtcgtcagggtctccctgaaaagaacgcaccaggaggagc gcacgcgtatgaccgtatgtcggaaagcaccagctcggaaagagggtggagagctgggtcaacgtcaga aaacagttcgtactctggagtgccgtccaccggggacggagagcaagcctacatactgttt	17+36 bp extra	358 aa, with the last 15 new
>V2 gatgtggagttacaatgcgttagccggaaacgacaaaggaaacagtacgtatgtccgttggtggacgtt tatgtatccataccataccagattccgtacaagcttcatggcgagaaccctcagaacacaccaggcacc cgtcatcaccacgcacacgcaggactacatcagcggacttgtggacttgtggcgctaaacgcacag aagggtgcatttataccctcaagccctcaacggcgacgcgcggtaaaggcagaacttccgtttttatc agtaagcctcgatcatagatcatggggcccactgtggatggacagggtgcactgtgtggctgaaggctacc gccccccatcaatgttactactgcgagaagcatgtcgtcagggtctccctgaaaagaacgcaccagg gaggagcgcagcgtatgaccgtatgtcggaaagcaccagctcggaaagagggtggagagctgggtcaa cgtcagaaaacagttcgtactctggagtgccgtccaccggggacggagagcaagcctacatactgttt	lacks 124 bp of exon 6, 36 bp extra	311 aa, with the last 9 new
>V3 gatgtggagttacaatgcgttagccggaaacgacaaaggaaacagtacgtatgtccgttggtggacgtt tatgaacggggtttatcagctcagcagaatccacaacagaaccctcacgtccgtcgccgacagttgt ctctgaacgttagcatggaggcgatccaaagccgcgcgcctctggacttgtctgttactccaataact acatcagcggacttgtggctggcgccaaacgcacagaagggtgcatttataccctcaagccctcaacg gcgcgcgcggtaaaggcagaacttccgttttatcgtaaagcctcgatcatagatcatggggccc agtggatggcagggtgcactgtgtggctgaaggctaccctgccccccatcaatgttactactgcgagaa gcatgtcgtcagggtctccctgaaaagaacgcaccaggaggaggcgcgcgtatgaccgtatgtcgg aaggcaccagctcggaaagagggtggagagctgggtcaacgtcagaaaacagttcgtactctggagtg cgccaccggggacggagagcaagcctacatactgttt	17 bp extra, lacks 63 bp of exon 6	354 aa, with the last 11 new
>V4 gatgtggagttacaatgcgttagccggaaacgacaaaggaaacagtacgtatgtccgttggtggacgtt tatgaacggggtttatcagctcagcagaatccacaacagaaccctcacgtccgtcgccgacagttgt ctctgaacgttagcatggaggcgatccaaagccgcgcgcctctggacttgtctgttactccaataagt gctccctcaaaagaacgcaccaggaggaggcgcgcgtatgaccgtatgtcggaaagcaccaggctcg ggaagagggtggagagctgggtcaacgtcagaaaacagttcgtactctggagtgccgtccaccggggac ggagagcaagcctacatactgttt	17 bp extra, lacks 63 bp of exon 6 and complete exons 7 and 8	364 aa, with the last 21 new
>V5 gatgtggagttacaatgcgttagccggaaacgacaaaggaaacagtacgtatgtccgttggtggacgtt tatgtatccatcgcgttgcggctcaagcgcacagaagggtgcatttataccctcaagcc caacggcgacgcggcgtaaaggcagaacttccgttttatcgtaaagcctcgatcatagatcatgg ggcccgatggatggcagggtgcactgtgtggctgaaggctaccctgccccccatcaatgttactactgc agaagcgtgtcgtcagggtctccctgaaaagaacgcaccaggaggaggcgcgcgtatgaccgtatgt cgggaaagcaccagctcggaaagagggtggagagctgggtcaacgtcagaaaacagttcgtactctggag tgcgtccaccggggacggagagcaagcctacatactgttt	lacks complete exon 6	307 aa, with the last 5 new
>V6 gatgtggagttacaatgcgttagccggaaacgacaaaggaaacagtacgtatgtccgttggtggacgtt tatgtgtccctgaaaagaacgcaccaggaggaggcgcgcgtatgaccgtatgtcggaaagcaca ggttcggaaagagggtggagagctgggtcaacgtcagaaaacagttcgtactctggagtgccgtccacc ggacggagagcaagcctacatactgttt	lacks complete exons 6, 7, and 8	317 aa, with the last 15 new

^aForward primer in exon 5 and reverse primer in exon 9 of *kita* were used for amplification; primer sequences are given in MATERIALS AND METHODS.